STABILITY UNDER ACIDIC AND BASIC CONDITIONS

Report

STABILITY UNDER ACIDIC AND BASIC CONDITIONS

CONTENTS

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Stability Under Acidic and Basic Conditions

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).

EC Commission Directive 1999/11/EC of 8 March 1999 (Official Journal No. L 77/8), as amended by EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No. L 50/44).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

These principles of Good Laboratory Practice are accepted by the regulatory authorities of the United States of America and Japan on the basis of intergovernmental agreements.

QUALITY ASSURANCE STATEMENT

Stability Under Acidic and Basic Conditions

The following inspections and audits have been carried out in relation to this study:

Study Phase	Date(s) of Inspection	Date of Reporting to Study Director and Management
Protocol Audit	5 April 2004	5 April 2004
Report Audit	17-18 June 2004	18 June 2004

Process based inspections: At or about the time this study was in progress inspections of procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated below:

Process Based Inspections	Date(s) of Inspection	Date of Reporting to Management
Chromatography	21 January 2004	21 January 2004
Spectrophotometry	15 April 2004	15 April 2004

In addition, an inspection of the facility where this study was conducted was carried out on an annual basis. These inspections were promptly reported to Company Management.

RESPONSIBLE PERSONNEL

Stability Under Acidic and Basic Conditions

The following staff member has reviewed this report.

SUMMARY

A study was performed to examine the stability of under acidic and basic conditions to satisfy a requirement of Korean polymer notification. The testing was based on the Japanese Polymer Flow Scheme, Polymer Commission, 1985 (page 840, Section IV, 1.2 Tests for Physicochemical Stability and Solubility under acid and alkaline conditions).

The stability of the test substance was investigated in a series of buffer solutions covering the pH range 1.2 to 9. Selected parameters (appearance, GPC and IR spectrum) were examined before and after periods of incubation in the respective buffer solutions, in order to assess the stability of ignificant changes were apparent at pH 1.2 and 4, but the polymer appeared to remain largely unchanged at pH 7 and 9.

was found to be stable under basic conditions, but unstable under acidic conditions.

INTRODUCTION

This study was designed to examine the stability of [redacted] ıder acidic and basic conditions to satisfy a requirement of Korean polymer notification. The testing was based on the Japanese Polymer Flow Scheme, Polymer Commission, 1985 (page 840, Section IV, 1.2 Tests for Physicochemical Stability and Solubility under acid and alkaline conditions).

The experimental start and completion dates were 1[redacted] 2004 respectively.

Location of study

TEST SUBSTANCE

STABILITY UNDER ACIDIC AND BASIC CONDITIONS

METHOD

The stability of the test substance was investigated in a series of buffer solutions covering the pH range 1.2 to 9. Selected parameters were examined before and after periods of incubation in the respective buffer solutions, in order to assess the stability of

PROCEDURE

Preparation of buffer solutions

pH 1.2 : Hydrochloric acid (1M aqueous, 32.2 ml) and potassium chloride (1M aqueous, 25 ml) were mixed and diluted to 500 ml with purified water. The pH was adjusted to 1.2 with 1M hydrochloric acid.

pH 4.0 : Disodium hydrogen orthophosphate dodecahydrate (6.36g) and potassium dihydrogen orthophosphate (1.50g) were dissolved in 500 ml of purified water. The pH was adjusted to 4.0 with orthophosphoric acid.

pH 7.0 : Sodium hydroxide (1M aqueous, 14.8 ml) and potassium dihydrogen orthophosphate (0.5M aqueous, 50 ml) were mixed and diluted to 500 ml with purified water. The pH was adjusted to 7.0 with orthophosphoric acid.

pH 9.0 : Sodium hydroxide (1M aqueous, 10.7 ml), potassium chloride (1M aqueous, 25 ml) and boric acid (0.5M aqueous, 50 ml) were mixed and diluted to 500 ml with purified water. The pH was adjusted to 9.0 with 1M hydrochloric acid.

Stability test

The test substance was placed in a mortar, made brittle by the application of liquid nitrogen and then ground to a white crystalline powder using a pestle. For each pH, two portions (approximately 200 mg) of ground test substance were accurately weighed into separate vessels and 100 ml of the appropriate buffer solution added. The vials were shaken at 40°C in a waterbath (24 hours for the pH 1.2 samples, 14 days for the pH 4.0 to 9.0 samples), and then filtered under vacuum through grade 3 sintered glass filter crucibles. As the quantities collected in the crucibles were relatively low, the filtrates were re-filtered through membrane filters (0.2 to 0.45 μm), and any material collected was bulked with that in the crucible. The filter cakes were dried to constant weight under vacuum at ambient temperature.

A portion (approximately 10 to 20 mg) of each dried sample was dissolved in tetrahydrofuran (1 ml) for analysis by gel permeation chromatography (GPC). Samples of Synocure 892 BA 70 which had not been incubated with buffer solution were similarly treated. Polystyrene standard solutions were analysed at the start and end of the chromatographic run to confirm that the retention time did not change significantly over the course of the run.

The infrared (IR) absorption spectrum of each of the dried samples was also recorded, as a thin film on a potassium bromide plate, over the range 4000 to 500 cm^{-1}. These were compared with the IR spectrum of Synocure 892 BA 70 which had not been incubated with buffer solution. At pH 7 and pH 9, only one spectrum was obtained as the labelling on the second replicate in each case had become unclear.

The pH of each filtrate was recorded.

GPC conditions

Instrument:	Hewlett Packard HP 1050 Series
Column:	PLgel 5µm 1000 Å and 500 Å in series (both 30 cm x 7.5 mm internal diameter)
Mobile phase:	Tetrahydrofuran
Column temperature:	35°C
Flow rate:	1 ml/min
Injection volume:	50 µl
Detector:	Pye Unicam PU 4026 Refractive Index detector

RESULTS

At each pH, the appearance of the dried filter cakes was not dissimilar to test substance which had not been incubated with buffer solution, although direct comparison was not possible due to the small quantities collected. The appearance of the pH 1.2 and 4 samples in solution was, however, significantly different from that of the pH 7 and 9 samples. The former samples were distinctly turbid, indicating possible reaction, whereas the latter samples remained as an unreacted mass in a clear solution.

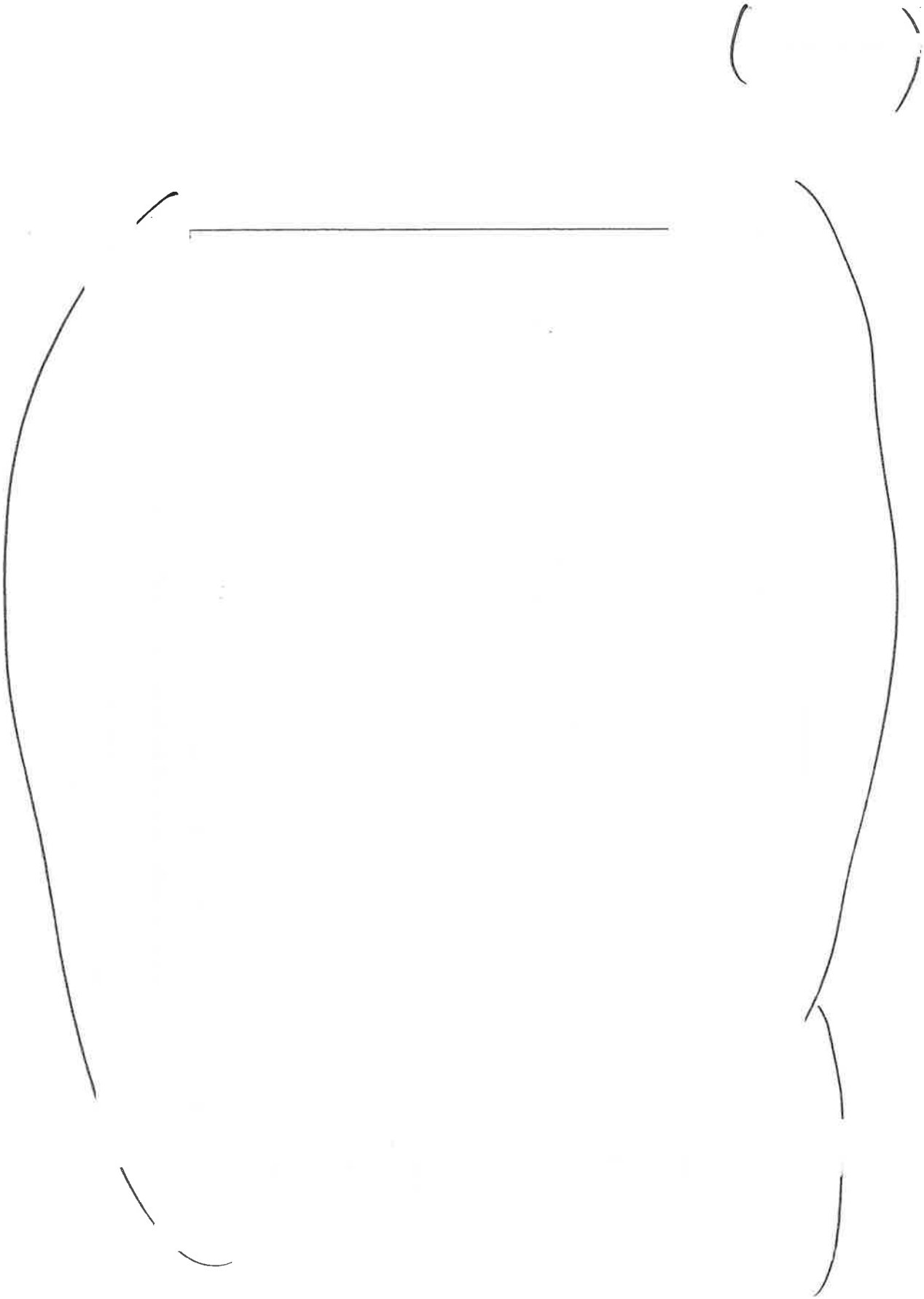
Figures 1 to 5 show example GPC chromatograms of fore and after the respective periods of incubation. It is evident from the pH 1.2 and 4 chromatograms that the parent polymer has broken down, as the elution profile is significantly different from that of the starting material. In GPC, higher molecular weight species elute first. At pH 7 and 9, there was no significant change in the elution profile.

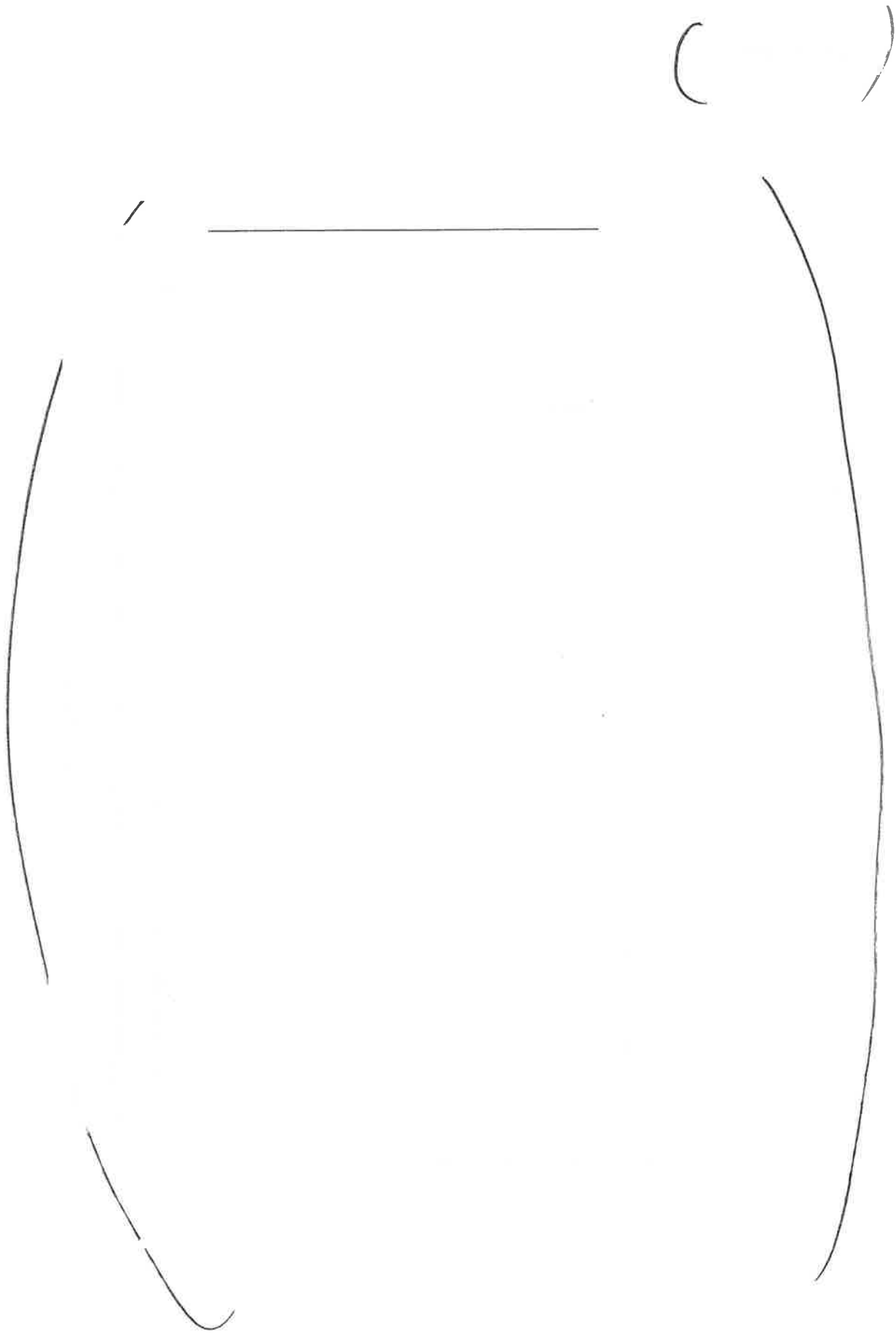
Figures 6 to 10 show example IR spectra o efore and after the respective periods of incubation. Although not conclusive, there is evidence of instability in pH 1.2 and 4 samples, as indicated by additional bands in the region of 3500 cm^{-1} functionalities produce bands in this region, and such would result from the hydrolysis of the ester groups of the polymer. The spectra are, however, noisy in this region, and this is therefore a tentative observation.

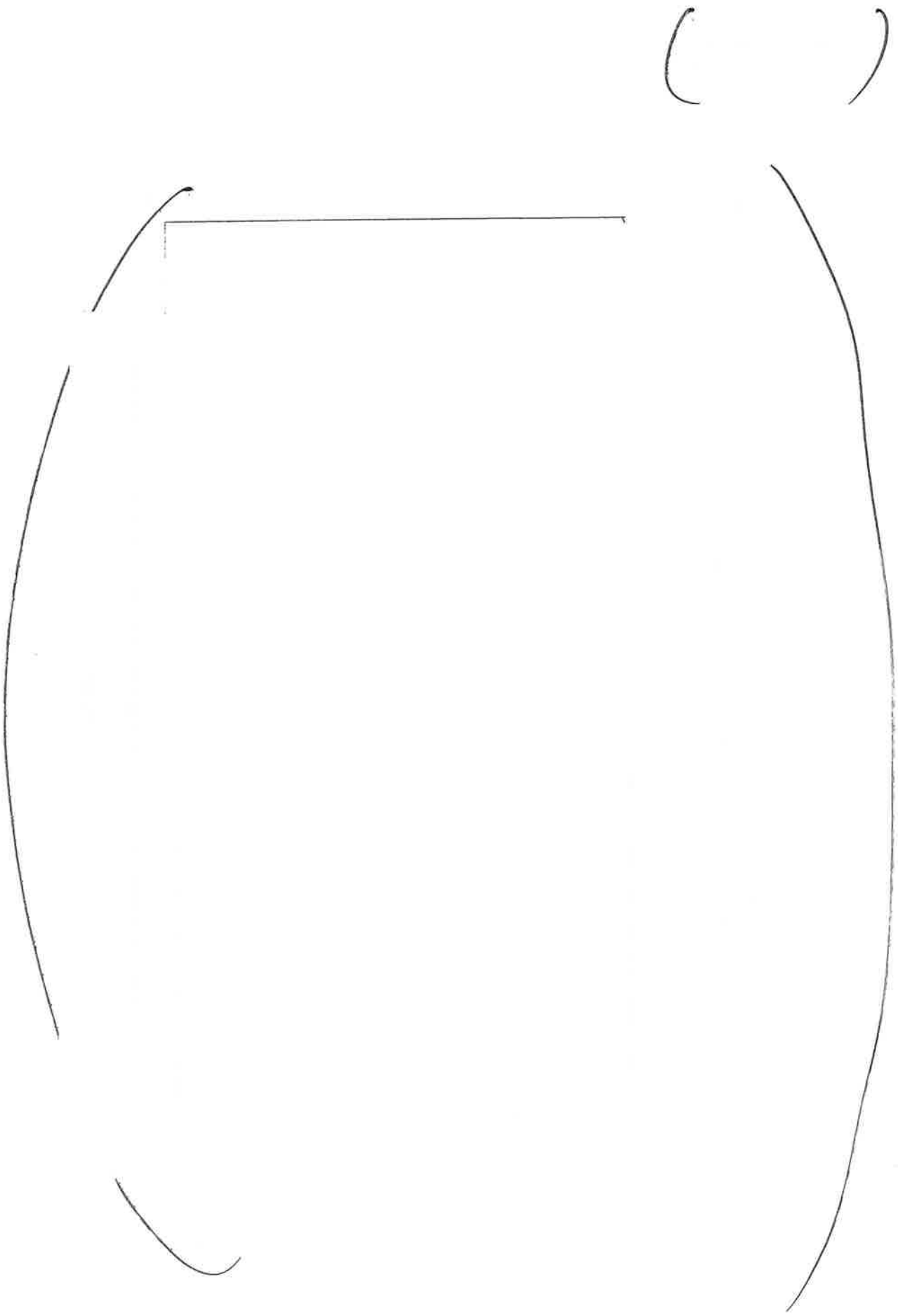
The results and observations indicate that is stable under basic conditions, but unstable under acidic conditions.

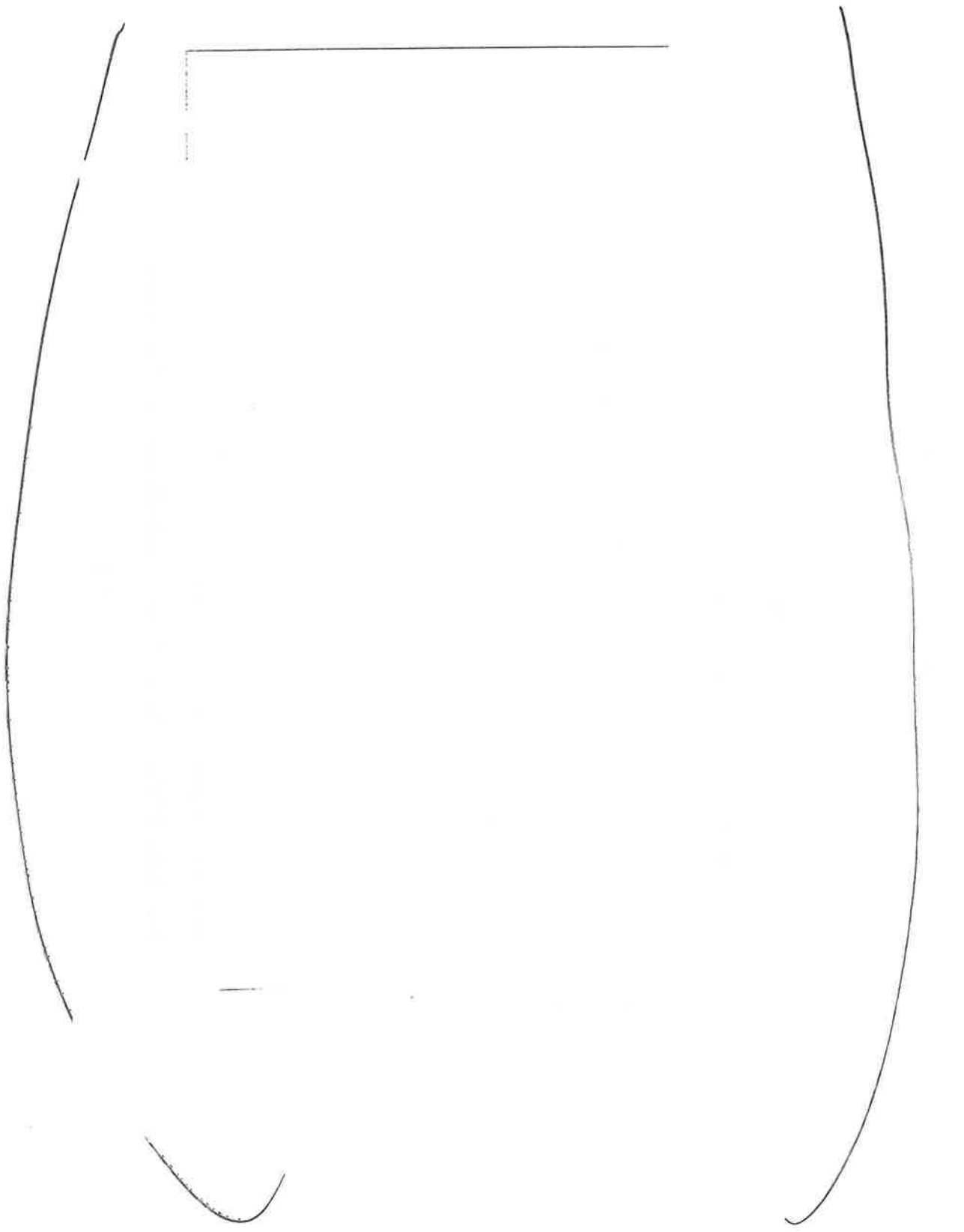
CONCLUSION

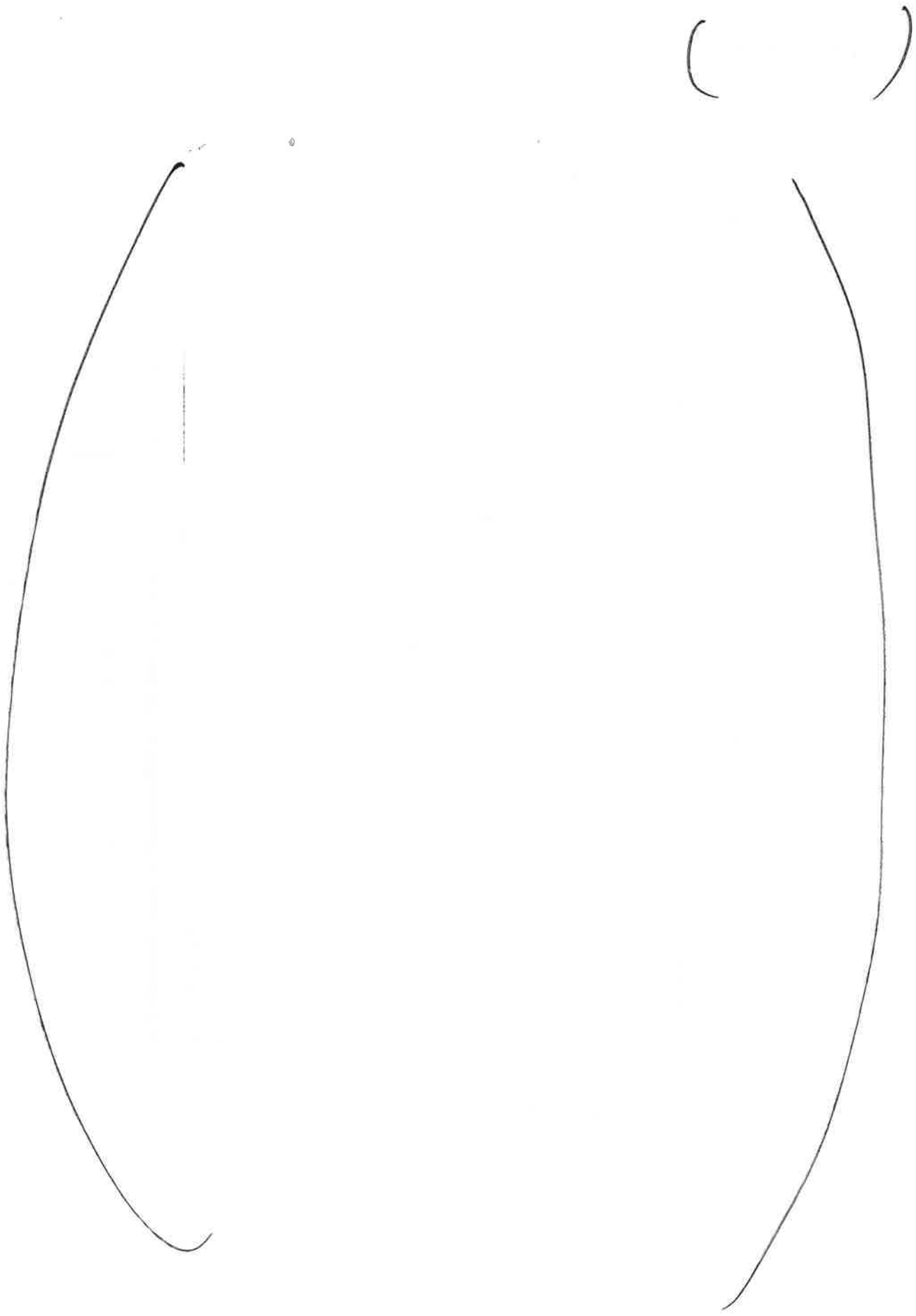
was found to be stable under basic conditions, but unstable under acidic conditions.

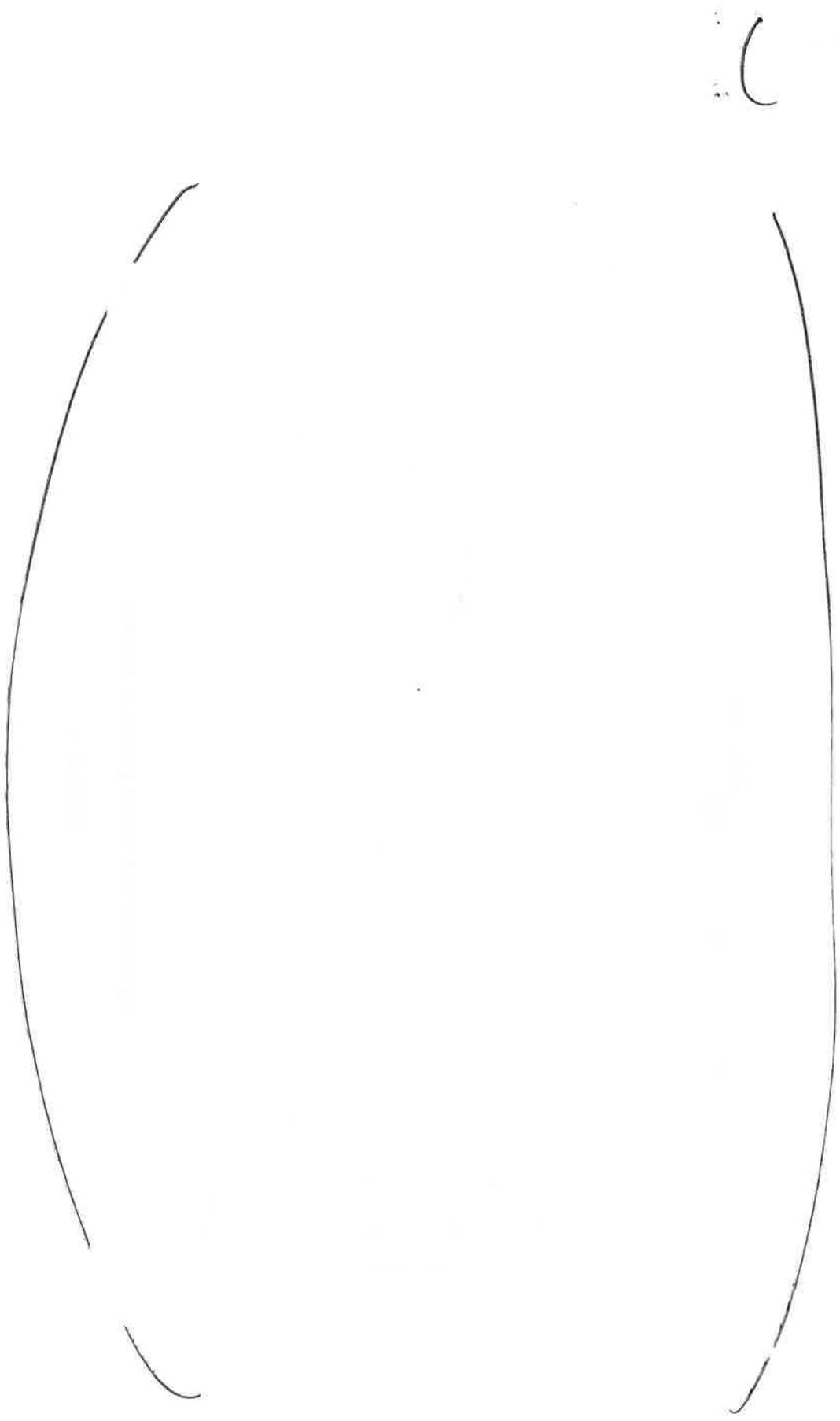


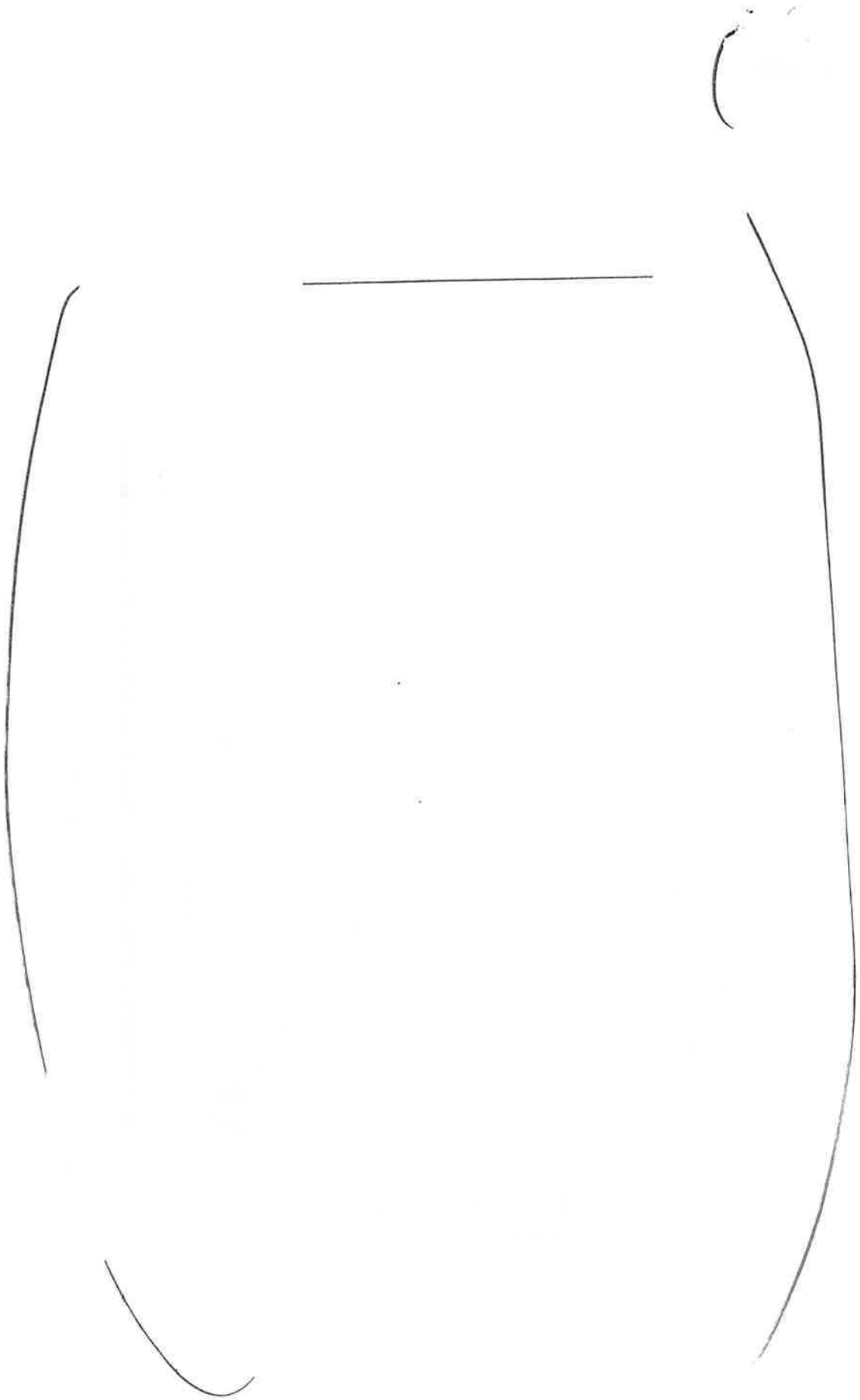


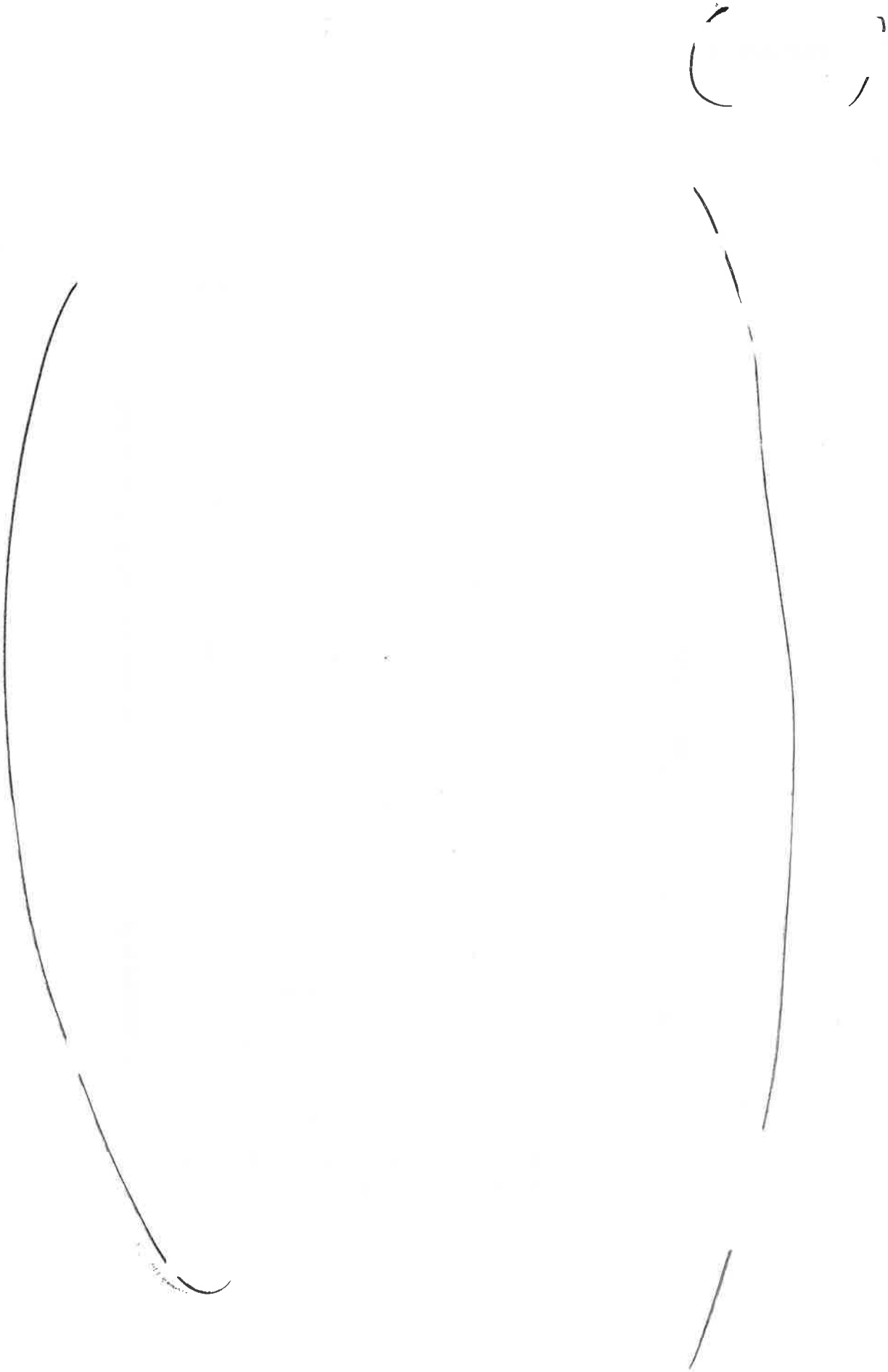


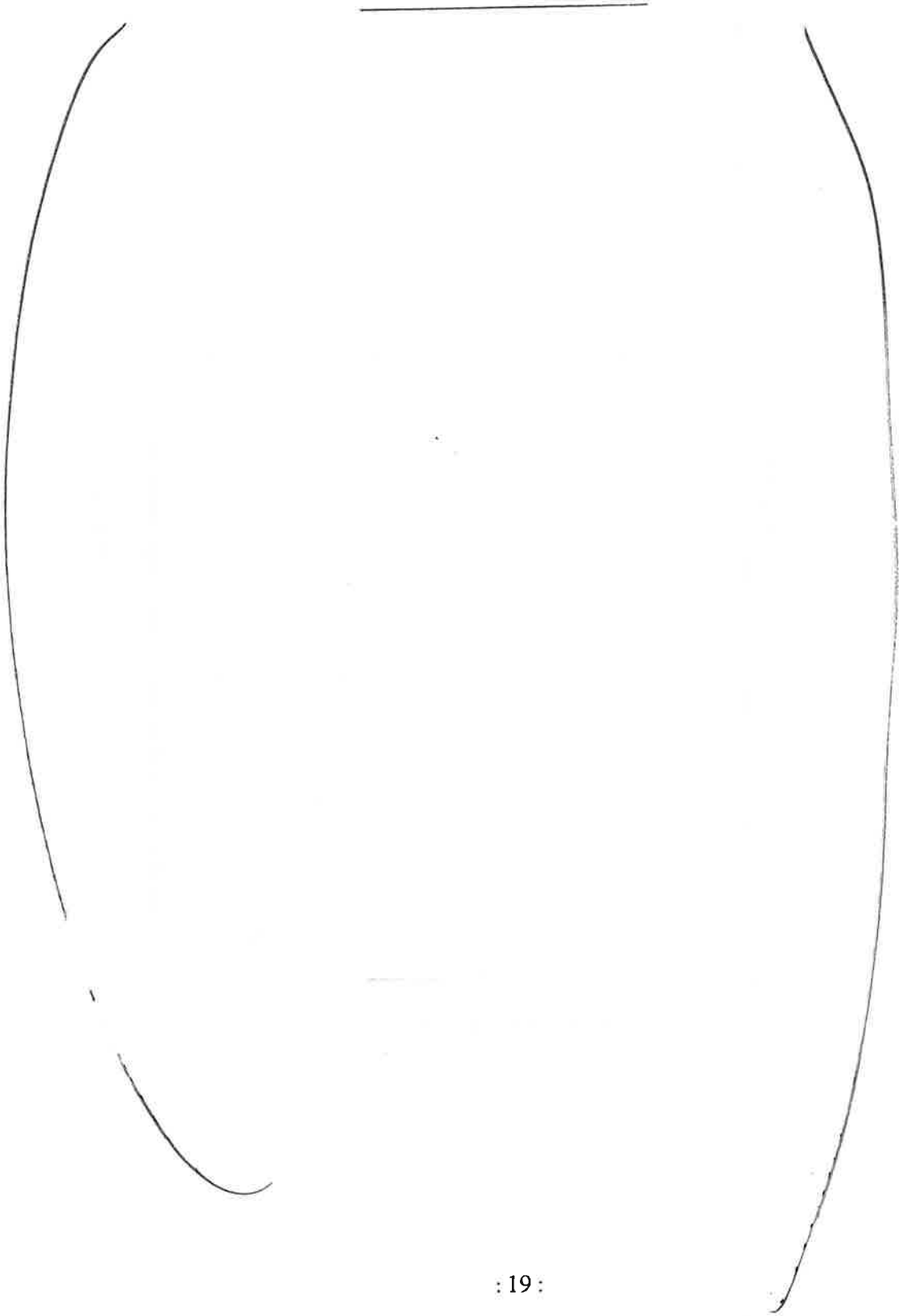


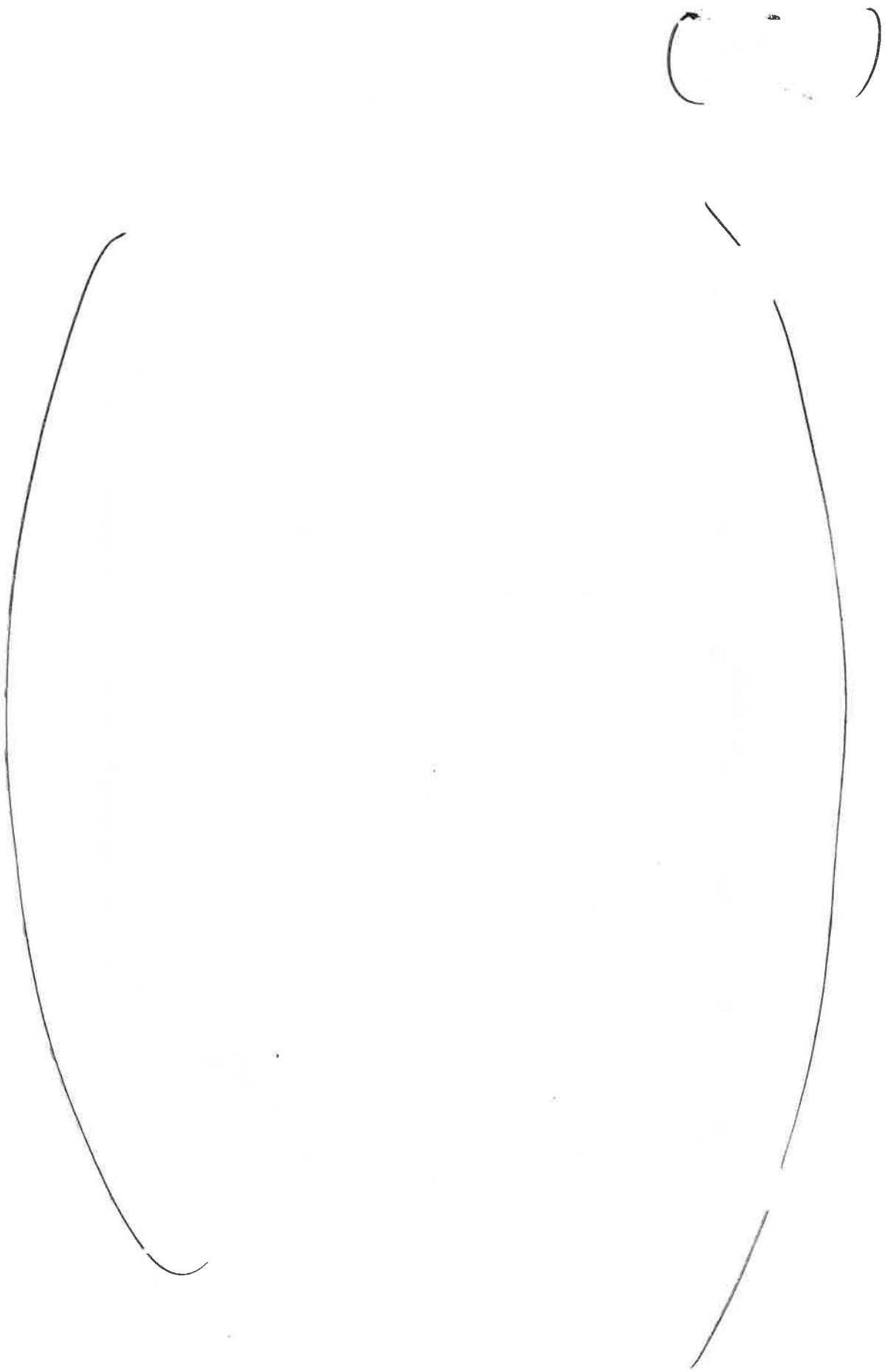












APPENDIX 1

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

TEST TYPE

Analytical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Mutagenicity
Toxicology
Phys/Chem Tests

DATE OF INSPECTION
22nd April 2003

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
25/7/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority